CHAPTER 9

EXTRACELLULAR MATRIX: TISSUE-SPECIFIC REGULATOR OF CELL PROLIFERATION

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INTRODUCTION

There are two broad categories of extracellular matrix (ECM) in tissues: interstitial/stromal matrix and basement membrane (BM). The interstitial matrix is the loose material around the epithelial cells that is separated from the cells by a basement membrane in many solid tissues. BM is most commonly found lining epithelial cell layers in tissues such as skin and breast (Fuchs et al., 1997; Ronnov-Jessen et al., 1996). Both the composition and the ultrastructure of BM exhibit tissue specificity, as well as temporal regulation during development (Jones and Jones, 2000; Miosge, 2001; Streuli, 1999; Tsai, 1998). Studies of the ultrastructural composition of basement membranes in vivo suggest that the relative arrangement of various ECM components are not only tissue specific but can also be different in certain parts of the same tissue (Lin and Bissell, 1993; Miosge, 2001). For example, ultrastructurally identical basement membranes, such as those found in the proximal and distal tubules of the kidney, have been shown to have a different molecular arrangement when examined by electron microscopy that allows observation of component orientation in tissue samples (Miosge, 2001).

One manifestation of tissue specificity is observed in the form of gene expression patterns, including expression of genes involved in cell cycle regulation. Establishment of tissue-specific gene expression patterns is not simply a result of which ECM molecules surround the cells in the adult tissue. Developmental processes (both during embryogenesis and postbirth, as is the case for the mammary gland) that produce a differentiated tissue involve sequential and interrelated gene regulatory

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events that are temporally regulated and that result in an integrated pattern of gene expression in the differentiated tissue (Bissell et al., 1999; Boudreau and Jones, 1999; Lohi et al., 1997; Wiseman and Werb, 2002). Therefore, to fully solve the puzzle of how ECM affects tissue-specific gene expression or cell cycle progression, the information context needs to include the history of the cell and its surrounding ECM (both of which change during development), as well as of the molecular characteristics of the cell-ECM interactions (Bissell and Ram, 1989; Werb and Chin, 1998; Wiseman and Werb, 2002).

Developmental events create a particular imprint in each tissue of both specific ECM molecules and ECM receptors. Thus how a cell behaves in response to its surrounding ECM is dependent not only on the level and composition of the ECM, but also on the cell-surface receptors that recognize and respond to it. The best-studied ECM receptors are the integrin family (Hynes, 1987, 1992; Miranti and Brugge, 2002). However, increasingly other receptors such as syndecans and dystroglycan have been shown to play a role in ECM-mediated signaling (Carey, 1997; Couchman and Woods, 1999; Rapraeger, 2000; Zimmermann and David, 1999). Multiple receptors can recognize a single type of ECM molecule, and a single receptor type may respond to multiple ECM components (Ashkenas et al., 1996; Boudreau and Jones., 1999; Watt, 2002). In addition how a cell responds to ECM is dependent on its growth factor and cytokine context. This is due to the extensive and reciprocal cross-talk between ECM, growth factor, and cytokine receptors (Damsky and Werb, 1992; Danen and Yamada, 2001; Schwartz et al., 1995). Besides cell cycle progression and differentiation, cell-ECM interactions regulate other cellular events such as apoptosis (Boudreau et al., 1995; Howlett et al., 1995). Not surprisingly, disruption of cell-ECM interactions, either by misregulated receptor function or by aberrant ECM composition and arrangement, results in tumorigenesis (Bissell and Radisky, 2001; Radisky et al., 2002; Simpson et al., 1994; Sternlicht et al., 2000; Sternlicht et al., 1999; Talhouk et al., 1992).

TISSUE SPECIFICITY OF ECM AND ITS RECEPTORS

Tissue-specific effects of ECM on cell proliferation are dependent on the molecular composition of the matrix surrounding the cells, as well as the ECM receptor makeup of the particular cell type within a tissue. Here we briefly discuss the function of some of the main ECM component families and their receptors with emphasis on tissue-distribution and tissue-specific diseases associated with these molecules. The ECM components we focus on are collagens, laminins, nidogens, glycosaminoglycans, and proteoglycans; ECM receptors include integrins, dystroglycan, and syndecans. Examples of genetic diseases associated with aberrant ECM or ECM receptor components are listed in Table 9.1.

TABLE 9.1. ECM Component Mutations in Human Disease

Gene[a]	Disease[b]	Reference[c]
Laminins		
LAMA2	Merosin-deficient muscular dystrophy	Kuang et al. (1998)
LAMA3	Herlitz type junctional epidermolysis bullosa (skin)	Vidal et al. (1995)
LAMB3	Herlitz type junctional epidermolysis bullosa (skin)	Kon et al. (1998)
LAMC3	Herlitz type junctional epidermolysis bullosa (skin)	Kon et al. (1998)
Collagens (fibrillar)		
COL1A1	Osteogenesis imperfecta (bone, muscle)	Ward et al. (2001)
COL1A2	Osteogenesis imperfecta (bone, muscle)	Trummer et al. (2001)
COL1A2	Ehlers-Danlos syndrome (connective tissue)	Byers et al. (1997)
COL1A2	Marfan syndrome (bone, ocular, cardiovascular)	Dalgleish et al. (1986)
COL2A1	Spondyloepiphyseal dysplasia (bone, retina)	Tiller et al. (1995)
COL2A1	Kniest dysplasia (bone, cartilage)	Spranger et al. (1994)
COL2A1	Achondrogenesis-hypochondrogenesis (bone, cartilage)	Godfrey and Hollister (1988)
COL2A1	Osteoarthiritis with mild condrodysplasia (bone, joint)	Ritvaniemi et al. (1995)
COL2A1	Stickler syndrome (joint, hearing, eye, cleft)	Brown et al. (1995)
COL2A1	Wagner syndrome (eye)	Richards et al. (2000)
COL3A1	Ehlers-Danlos syndrome (connective tissue)	Smith et al. (1997)
COL3A1	Familial aortic aneurysms (endothelial)	van Keulen et al. (1999)
COL6A1	Bethlem myopathy (muscle)	Jobsis et al. (1996)
COL6A2	Bethlem myopathy (muscle)	Jobsis et al. (1996)
COL6A3	Bethlem myopathy (muscle)	Jobsis et al. (1996)
COL10A1	Metaphyseal chondrodysplasia (bone)	McIntosh et al. (1995)
COL10A1	Spondylomethaphyseal dysplasia (bone)	Ikegawa et al. (1998)
COL11A1	Stickler syndrome (joint, hearing, eye, cleft)	Snead et al. (1996)
COL11A1	Marshall syndrome (hearing, eye, facial skeletal defect)	Meisler et al. (1998)
Collagens (BM)		
COL4A3	Alport syndrome (kidney)	Kashtan (1995)
COL4A3	Benign hematuria (kidney)	Badenas et al. (2002)
COL4A4	Alport syndrome (kidney)	Kashtan (1995)
COL4A4	Benign hematuria (kidney)	Lemmink et al. (1996)
COL4A5	Alport syndrome (kidney)	Kashtan (1995)
COL4A6	Alport syndrome (kidney)	Kashtan (1995)
COL8A2	Fuchs endothelial corneal dystrophy	Biswas et al. (2001)

[a] A gene is listed more than once if it has multiple associated diseases. These genes were selected because mutations in them are associated with genetic diseases.

[b] The affected tissues are listed in parentheses.

[c] Additional information and references are available at the NIH web site OMIM (Online Mendelian Inheritance In Man), at *http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=OMIM.*

Collagens

Collagens are the most abundant components of the extracellular matrix as well as of interstitial/stromal matrices. Indeed, collagens are the most abundant proteins in mammals, being a major component of the skin and bone. Most collagens are heterotrimeric molecules of alpha chains (more than 25 of which have been described so far) folded into a coiled-coil triple helix. The triple helix can be homotrimeric, as in the case of type II, type III, type IV, and type VII. Collagens can also be heterotrimeric containing either two or three different alpha chains. For example, collagen type XI is heterotrimeric, containing an alpha 1 (COL11A1), an alpha 2 (COL11A2), and an alpha 3 (COL11A3) chain; collagen type I contains two alpha 1 chains (COL1A1) and one alpha 2 chain (COL1A2). More than 20 different types of collagens have been described based on the structural domains of their alpha chain components. However, within one collagen type, there may be significant variation among molecules because of the large number of alpha chain isoforms that exist. For example, the basement membrane collagen, collagen type IV, can be composed of helix combinations that are compiled from six different gene products (COL4A1 through COL4A6), and their many alternatively spliced variants, in a somewhat tissue-specific manner (Myllyharju and Kivirikko, 2001).

Collagens can be organized into fibrillar structures in connective tissues and in interstitial matrices of soft tissues, or they organize into sheet-like structures in basement membranes. Some nonfibrillar collagens are found associated with fibrils, and sometimes also with the basement membrane and are thought to stabilize interactions between the basement membrane and the interstitial stromal matrix. Fibril-forming collagens include collagen types I, II, III, V, VI, IX, X, and XI (Kadler, 1994, 1995). Fibril-associated collagens are collagen types VII, XII, XV, XVI, XIX, and XXI. Basement membrane collagens are collagen types IV and VIII (Fukai et al., 1994). Functions of collagens XIII, XIV, XXII, XXIII, and XXVI have not been well characterized. Collagen type XVII is an unusual collagen since it is a transmembrane protein (Uitto and Pulkkinen, 1996).

A comprehensive overview of the tissue-specific distribution and functions of this vast number of collagens is not within the scope of this review. However, examples below indicate that certain tissues feature some collagens more prominently, and that diseases associated with each collagen type hints at the tissue specificity of expression (Olsen, 1995; Tryggvason, 1995). Examples of tissue-specific functions and diseases of fibrillar collagen are as follows: Collagen type I is a fibrillar collagen, most commonly found in connective tissues such as bone, cartilage, and skin (Cremer et al., 1998). Mutations in collagen type I have been associated with bone diseases such as osteogenesis imperfecta, Ehlers-Danlos syndrome, and idiopathic osteoporosis, as well as causing a particular type of skin tumor called dermafibrosarcoma protuberans (Kuivaniemi et al., 1997). Collagen type II is a fibrillar collagen found in cartilage and in the vitreous

humor of the eye (Cremer et al., 1998; Kuivaniemi et al., 1997). Accordingly mutations in collagen type II are associated with connective tissue disorders such as achondrogenesis, chondrodysplasia, early onset familial osteoarthritis, SED congenita, Langer-Saldino achondrogenesis, Kniest dysplasia, Stickler syndrome type I, and spondyloepimetaphyseal dysplasia Strudwick type. Collagen type III is also a fibrillar collagen that is associated with flexible connective tissues such as that of the skin, lung, and vasculature, often found associated with collagen type I. Accordingly mutations in this collagen are found associated with blood vessel abnormalities, such as aortic and arterial aneurysms, as well as with connective tissue disorders such as Ehlers-Danlos syndrome (Kuivaniemi et al., 1997). Type V collagen is found in tissues containing type I collagen and appears to regulate the assembly of heterotypic fibers composed of both type I and type V collagen. Collagen VI is a major structural component of muscle microfibrils, and mutations in the genes that code for the collagen VI subunits result in the autosomal dominant disorder, Bethlem myopathy. Type X collagen is short fibrillar collagen that is expressed by chondrocytes during ossification. Mutations in collagen type X result in bone diseases such as Schmid-type metaphyseal chondrodysplasia (SMCD) and Japanese-type spondylometaphyseal dysplasia (SMD) (Kuivaniemi et al., 1997). Collagen type XI is a fibrillar collagen which is a minor constituent of cartilage and mutations in this collagen are associated with type II Stickler syndrome and with Marshall syndrome, both connective tissue disorders (Cremer et al., 1998; Kuivaniemi et al., 1997).

Examples of tissue-specific functions and diseases of basement membrane collagens are as follows: Collagen type IV is found in many basement membranes. There are 6 alpha chains that can give rise to a possible 56 combinations of collagen type IV. Furthermore there are multiple isoforms of many of the subunits, providing a large number of possible collagen type IV combinations for establishment of tissue specificity (Kuhn, 1995; Petitclerc et al., 2000). Mutations in the alpha 1 chain of collagen type IV are associated with type II autosomal Alport syndrome (hereditary glomerulonephropathy) and with familial benign hematuria (thin basement membrane disease). Both diseases affect the kidneys, suggesting that the alpha 3, alpha 4, alpha 5 chains (COL4A3, COL4A4, COL4A5) are prominently featured in the kidney basement membranes (Kashtan, 2000). Type VIII collagen is the main component of the corneal epithelium. This collagen has only two alpha subunits but it can exist either as a homo- or a heterotrimer, as a combination of these two subunits. Mutations in collagen type VIII cause corneal endothelial dystrophy, consistent with the specificity/prominence of its function in the cornea (Meek and Fullwood, 2001).

Laminins

Laminins are a family of heterotrimeric extracellular basement membrane glycoproteins. They are composed of three chains, alpha, beta, and gamma, which form a cruciform structure with three short arms (each

from a different chain) and a long arm (composed of all three chains) (Cheng et al., 1997; Yurchenco et al., 1992). Each laminin subunit has multiple functional domains and is encoded by a distinct gene. Expression of laminin encoding genes is regulated at multiple levels, giving rise to different isoforms. Laminin alpha, beta, and gamma chain isoforms can combine to give rise to different laminins. A total of 11 laminins have been described so far (and they were named in the order of their discovery: laminin-1, laminin-2, etc.); however, a very large number of permutations of the 5 alpha, 4 beta, and 3 gamma subunits and their isoforms is possible (Ekblom et al., 1998).

The tissue-specific distribution, and possible distinct functions of the different alpha, beta, and gamma chains and their isoforms remain largely unknown. Some laminin components have wide tissue distributions. For example, laminin beta 1 is expressed in most tissues that produce a basement membrane, and is one of the three chains constituting laminin 1 (alpha 1/LAMA1, beta 1/LAMB1, gamma 1/LAMC1). This was the first laminin isolated from Engelbreth-Holm-Swarm (EHS) tumor (a basement membrane gel isolated from EHS tumors is widely used in reconstitution experiments in culture and is referred to as a laminin-rich basement membrane, lrBM, in the rest of this chapter) (Friedman et al., 1989; Grant et al., 1985; Kleinman et al., 1982; Li et al., 1987). However, some chains are more prominent in certain tissues, reflecting tissue specificity of BM composition. For example, laminin alpha 2 (LAMA2) is prominently expressed in striated muscle (laminins that contain LAMA2 are called merosins), and the significance of LAMA2 function in muscle is exemplified by the causal relationship between mutations in this gene and congenital merosin-deficient muscular dystrophy (Kuang et al., 1998; Vachon et al., 1996; Wewer and Engvall, 1996). Laminin 5 (alpha 3/LAMA3, beta 3/LAMB3, gamma 2/LAMC2) has a significant role in skin keratinocyte function, as shown by the observation that mutations in any one of its three subunits causes Herlitz type junctional epidermolysis bullosa (Kon et al., 1998; Vidal et al., 1995). Another example of laminin chains that show much restricted tissue distribution is beta 2/LAMB2. It is enriched in the basement membrane of muscles at the neuromuscular junctions, kidney glomerulus and vascular smooth muscle (Hunter et al., 1989; Virtanen et al., 1995).

Nidogens/Entactins

Nidogens are sulfated glycoproteins that are found in basement membranes. Two mammalian nidogens have so far been identified, nidogen 1 and nidogen 2. Their function is to bridge the laminin network with the collagen IV network and provide stability of the basement membranes. Nidogen is essential for both embryonic development and maintenance of proper differentiation of adult tissues. Since there are only two members of the family, nidogen is a less likely candidate for establishment and maintenance of tissue specificity (Dziadek, 1995; Mayer et al., 1998; Timpl et al., 1984). However, distribution of the two members of this family does appear to show some tissue specificity: nidogen 2 is more

prominent in endothelial cells but nidogen 1 is more widely distributed (Schymeinsky et al., 2002).

Proteoglycans and Glycosaminoglycans

Proteoglycans have important structural, as well as signaling functions (Perrimon and Bernfield, 2001). They play a part in providing shape and biomechanical strength to organs and tissues mainly because of the nature of their glycosaminoglycan (GAG) chains. Structural diversity within GAG chains ensures that each protein-GAG interaction is as specific as necessary. A single proteoglycan, even if it carries a single GAG chain, can bind multiple proteins, suggesting the possibility of functional diversity (Delehedde et al., 2001). The core proteins of cell surface proteoglycans may be transmembrane, such as syndecan (an ECM receptor), or GPI-anchored, such as glypican, or matrix proteins, such as perlecan. Perlecan is a heparin sulfate proteoglycan and is a major component of basement membranes (Oldberg et al., 1990; Yanagishita, 1993).

Many growth factors and morphogens (fibroblast growth factors, hepatocyte growth factor/scatter factor, members of the midkine family, and wnts), and matrix proteins (collagen, fibronectin, and laminin) interact with proteoglycans when they signal. The GAG-protein interactions serve to regulate the signal output of growth factor receptor tyrosine kinases and hence cell fate (Park et al., 2000). In addition GAGs coordinate stromal and epithelial development, and they are active participants in mediating cell-cell and cell-matrix interactions (Kresse and Schonherr, 2001; Lander et al., 1996).

An example of a functionally diverse GAG chain (that actually is not attached to protein) is hyaluronic acid/hyaluronan (HA). HA serves a variety of functions, including space filling, lubrication of joints, provision of a matrix through which cells can migrate, and intracellular signaling (Toole, 2001). HA is actively produced during wound healing and tissue repair to provide a framework for growth of blood vessels and fibroblasts (Toole et al., 2002). In addition the interaction of HA with the leukocyte receptor CD44 is important in tissue-specific homing by leukocytes (Turley et al., 2002), and overexpression of HA receptors has been correlated with tumorigenicity and tumor metastasis (Isacke and Yarwood, 2002; Toole, 2002).

Integrins

Integrins are the most extensively studied receptors that transmit ECM signals. The name integrin stems from the observation that integrins are the integrators of extracellular (ECM) and intracellular (cytoskeletal) signals (Boudreau and Jones, 1999; Giancotti and Ruoslahti, 1999; Hynes, 1987). Integrins are heterodimeric receptors of alpha and beta subunits that interact noncovalently to form transmembrane receptors. Currently 17 α and 8 β subunits have been identified. So far, at least 20 heterodimeric receptors have been identified, many of which are tissue specific (Schwartz and Ingber, 1994; Schwartz et al., 1995). Substrates of

integrins are various ECM components including laminins, collagens, fibronectin, and vitronectin. A specific integrin heterodimer can have multiple substrates. For example, αvβ3 receptor shows strong binding affinity for fibronectin, collagen, tenscin-C, thrombospondin, and fibrinogen, as well as vitronectin. A certain type of ECM molecule can interact with more than one kind of integrin heterodimer in a cell context dependent manner (Giancotti and Ruoslahti, 1999). For example, laminin binds α3β1, α6β1, and α6β4 integrins with high affinity. The number of diseases that have been shown to have mutations in integrin subunits is limited so far. This could be partly because such mutations may be lethal. The most prominent defect identified so far is epidermolysis bullosa, a skin disease that is strongly associated with mutations in integrin beta 4 (ITGB4 gene) (Pulkkinen et al., 1998).

In addition to transmitting biochemical signals from ECM proteins, integrins are also involved in sensing and transducing mechanochemical signals (Alenghat and Ingber, 2002; Chen et al., 1997; Ingber, 2002). Integrin signaling has effects on cell adhesion, the cytoskeleton, proliferation, growth, differentiation, and apoptosis pathways. How these pathways are connected is discussed in some detail below, with emphasis on integrin-mediated signaling effects on cell cycle progression.

Non-integrin ECM Receptors

A number of transmembrane cell surface receptor families function in transmitting ECM mediated signals. Among them are syndecans and glypicans. In addition dystroglycan has recently been identified as an ECM signal transmitting receptor. Syndecans are expressed on cells that are dependent on adhesion for proliferation and their distribution is highly cell type and tissue specific. Syndecans are involved in formation

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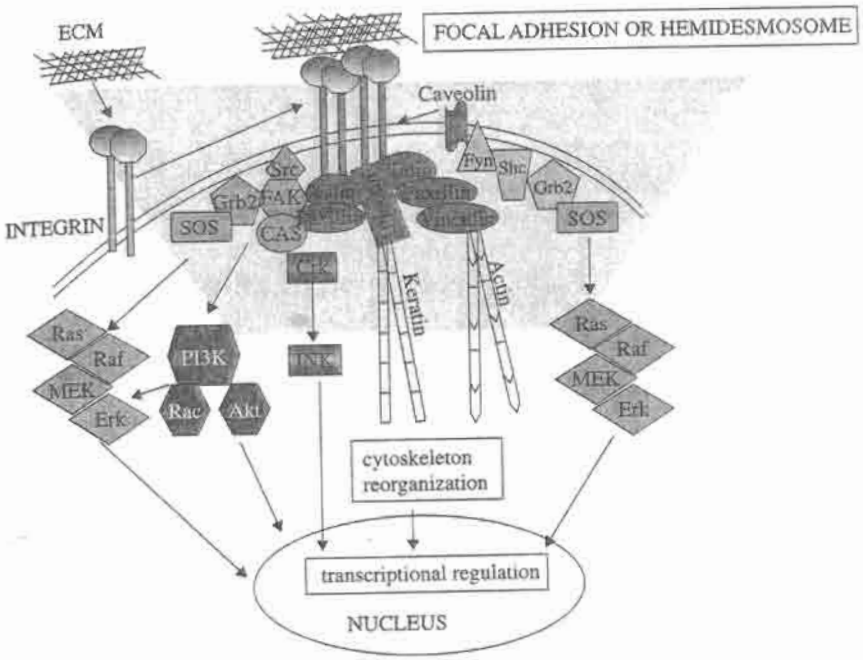
Figure 9.1. Integrin signaling exhibits dynamic reciprocity. **(A)** ECM-to-nucleus signaling. Integrins are activated and cluster either by crosslinking or occupancy of ECM molecules. Interaction of the cytoplasmic tail of integrins with talin, paxillin, and vinculin or plectin has two main consequences: the actin/keratin cytoskeleton is reorganized, and activation of focal adhesion kinase (FAK) or Shc initiates downstream signaling pathways, such as the Ras-Raf-MEK-ERK (MAPK cascade), the PI-3 kinase, and the JNK pathway. The clustered integrin/ECM and many downstream targets that accumulate at the adhesion site form a focal adhesion complex or a hemidesmosome. Downstream effects of the activation of the MAPK cascade, the PI-3 kinase, and the JNK pathway include transcriptional modulation of a number of genes including those involved in cell cycle regulation. **(B)** Nucleus-to-ECM signaling. The affinity of membrane-bound integrins for ECM can be altered by change in nuclear functions. Transcriptional changes result in altered expression of cell surface receptors such as integrins, as well as in the composition of ECM via regulation of ECM components and ECM-degrading enzymes (blue). These growth status regulated changes in the nucleus are initiated by the MAPK cascade, or G-protein, PtdIns(4,5)P2 and PKC. MAPKs and PKC signals can also affect the affinity of integrins for their ligands.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Ras
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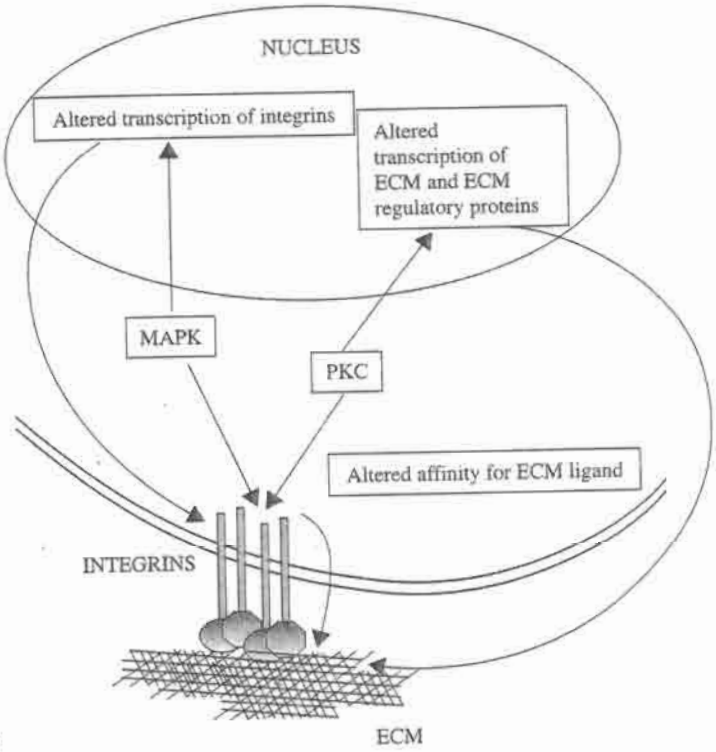
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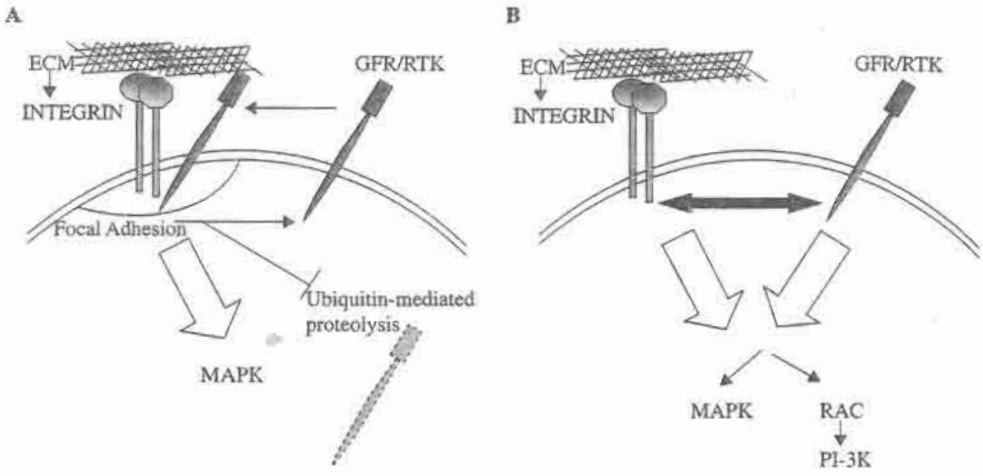


Figure 9.2. Cooperation of integrin and RTK/GFR signaling. **(A)** ECM activated integrin signaling results in focal adhesion complex formation followed by recruitment of growth factor receptors (GFRs). Such recruitment activates GFRs and their downstream targets such as the MAPK pathway. Alternatively, stability of GFRs can be increased by blocking their ubiquitin-mediated degradation upon attachment to ECM. **(B)** Sustained activation of the MAPK pathway, as well as activation of RAC-mediated signaling can be dependent on both integrin and GFR signaling.

of focal adhesion complexes, as are integrins (see below), and syndecans can also modulate integrin function (Carey, 1997; Couchman and Woods, 1999; Rapraeger, 2000; Zimmermann and David, 1999).

Glypicans are a distinct family of transmembrane heparan sulfate proteoglycans that contain a core protein anchored to the cell surface via a glycosyl phosphatidylinositol linkage (GPI-linked). Some members of the glypican family of integral membrane proteins are putative cell surface coreceptors for growth factors, ECM proteins, proteases, and antiproteases (Lander et al., 1996), and have been implicated in regulation of cell cycle progression (Delehedde et al., 2001).

Dystroglycan is both an ECM receptor and an organizer of the ECM (Ekblom et al., 1998; Henry et al., 2001). Originally isolated in skeletal muscle, it has now been shown to function in many tissues (Durbeej et al., 1998b). Dystroglycan substrates identified up to now include laminins, and proteoglycans. So far, only one dystroglycan gene has been identified. It has wide tissue distribution and multiple functions (Durbeej and Ekblom, 1997; Durbeej et al., 1998a,b; Muschler et al., 2002).

TISSUE-SPECIFIC EFFECTS OF ECM ON CELL PROLIFERATION IN MOUSE MODELS

Most knockout mouse models of ECM components and ECM receptors are not conditional; therefore the final phenotype observed reflects the

changes throughout development. For this reason, interpretations of such models for tissue specificity of ECM effects on cell cycle progression need to be made with caution. Furthermore ability of another ECM component to substitute for the mutated gene can obscure interpretations. However, using conditional loss-of-function mutations in mice allows observation of tissue-specific developmental defects for certain ECM or receptor components. Tissue-specific conditional knockouts in the adult mouse are the best sources of information for determining cell type specificity of ECM or its receptors in terms of their roles in cell cycle control. Only a few examples of conditional knockouts exist for ECM and its receptors, namely for β1 integrin and dystroglycan (Brakebusch et al., 2000; Hirsch et al., 1996; Raghavan et al., 2000). An overview of ECM and ECM receptor components that have been knocked out in mice is shown in Table 9.2.

Examination of homozygous knockouts of ECM components and their receptors shows that loss of a single subunit is usually not embryonic lethal, suggesting that other BM components can substitute for function in most tissues. Nidogen-1 and nidogen-2 knockout mice demonstrate an extreme case of this. Targeted homozygous disruption of neither nidogen-1 nor nidogen-2 results in the disruption of BM. Since nidogen is necessary for the integrity of basement membranes, this suggests that nidogen-1 and nidogen-2 can substitute for each other's function with no or very little tissue specific requirements (Murshed et al., 2000; Schymeinsky et al., 2002). In other ECM knockouts, some tissues and cell types show gross developmental abnormalities, some of which are due to changes in the regulation of cell proliferation by ECM. For example, disruption of the alpha 3 chain of laminin 5 result in abnormalities in the survival of epithelial cells (especially in the skin) as well as extreme blistering of the skin, similar to a skin disease known as junctional epidermolysis bullosa-gravis in humans. LAMA3 was shown to be necessary for the proper formation and stabilization of hemidesmosomes in the epidermis, and the skin abnormalities could be attributed to the hemidesmosome defects (Ryan et al., 1999). A few knockouts do show embryonic lethality, suggesting that the mutated genes are essential in early development and not replaceable by similar components. For example, mice homozygous null for the gamma 1 chain of laminin (LAMC1), beta 1 integrin (ITGB1), dystroglycan (DAG1) show embryonic lethality (Smyth et al., 1999; Stephens et al., 1995; Williamson et al., 1997). Conditional knockouts of integrin beta 1 and dystroglycan have been produced for multiple tissues (Brakebusch et al., 2000; Cohn et al., 2002; Hirsch et al., 1996; Moore et al., 2002; Raghavan et al., 2000). Conditional knockouts are more informative about cell proliferation effects especially when the knockout event occurs either after completion of the development of the tissue or in later stages of development. For example, disrupting beta 1 integrin in skin, using a keratin 14 promoter that is turned on very late in development, results in failure of basement membrane assembly and maintenance, and impairment of epidermal proliferation (Raghavan et al., 2000).

TABLE 9.2. ECM and ECM Receptor Mutations in Mice

Gene	Homozygous Mutant Phenotype	Reference
Laminins		
LAMA2	Murine muscular dystrophy	Xu et al. (1994)
LAMA3	Neonatal lethality	Ryan et al. (1999)
LAMA4	Nervous system developmental defects	Patton et al. (2001)
LAMB2	Aberrant neuromuscular development	Noakes et al. (1995)
LAMB3	Junctional epidermolysis bullosa (skin blistering)	Kuster et al. (1997)
LAMC1	Embyonic lethality	Smyth et al. (1999)
Collagens		
COLA1	Dermal fibrosis, impaired uterine involution	Liu et al. (1995)
COLA2	Glomerulopathy (kidney)	Phillips et al. (2002)
COL3A1	Cardiovascular defects	Liu et al. (1997)
COL4A3	Alport syndorome (kidney)	Cosgrove et al. (1996)
COL5A2	Spinal deformities, skin and eye abnormalities	Andrikopoulos et al. (1995)
COL10A1	Defects in bone development and hematopoiesis	Gress and Jacenko (2000)
COL11A1	Skeletal development defects	Li et al. (1995)
COL11A2	Hearing loss	McGuirt et al. (1999)
COL15A1	Skeletal myopathy, cardiovascular defects	Eklund et al. (2001)
Nidogens		
NID1	No detectable defects	Murshed et al. (2000)
NID2	No detectable defects	Schymeinsky et al. (2002)
Proteoglycans		
HSPG2	Cartilage defects, cardiac and brain BM defects	Costell et al. (1999)
AGC	Bone and cartilage phenotypes	Wai et al. (1998)
FN1	Mesoderm, neural tube, vascular development defects	George et al. (1993)
Integrins		
ITGA1	Derived MEFs have adhesion defects in vitro	Gardner et al. (1996)
ITGA2	Kidney and lung development defects	Kreidberg et al. (1996)
ITGA4	Placental and cardiac development defects	Yang et al. (1995)
ITGA5	Embryonic mesodermal defects	Yang et al. (1993)
ITGA6	Epidermolysis bullosa (skin blisters), neonatal death	Georges-Labouesse et al. (1996)
ITGA7	Homozygous null shows muscle and tendon organization defects, including a novel form of muscular dystropy	Mayer et al. (1997), Miosge et al. (1999)
ITGA8	Homozygous null has defects in kidney morphogenesis	Muller et al. (1997)
ITGA9	Homozygous mutants have fatal bilateral chylothorax (lymphatic system and thoracic duct defects)	Huang et al. (2000)
ITGAE	Cutaneous inflammatory disorder	Schon et al. (2000)
ITGAM	Neutrophil adhesion, migration, apoptosis defects	Coxon et al. (1996), Ding et al. (1999)
ITGAV	Lethality preceded by aberrant vasculogenesis, angiogenesis, and organogenesis	Bader et al. (1998)
ITGB1	Peri-implantation lethality and inner cell mass failure Their ES cells have migration and adhesion defects	Fassler et al. (1995), Stephens et al. (1995)

TABLE 9.2. *Continued*

Gene	Homozygous Mutant Phenotype	Reference
ITGB1	Conditional null in skin has epidermal proliferation, basement membrane formation, and hair follicle invagination defects	Brakebusch et al. (2000), Raghavan et al. (2000)
ITGB1	Chimeric mice, with null hematopoietic cells display impaired migration of hematopoietic stem cells	Hirsch et al. (1996)
ITGB3	Homozygous mutants of the cytoplasmic domain have impaired platelet function	Law et al. (1999)
ITGB3	Osteoclast abnormalities	McHugh et al. (2000)
ITGB3	Heterozygous mutants have placental defects and reduced survival	Hodivala-Dilke et al. (1999)
ITGB4	Homozygous nulls have defects in hemidesmosome formation, cell adhesion, and cell survival, followed by lethality soon after birth	Dowling et al. (1996), Frei et al. (1999)
ITGB4	Homozygous deletion of the cytoplasmic domain results in cell cycle and adhesion defects, and lethality postbirth	Murgia et al. (1998)
ITGB6	Juvenile baldness and asthma-like syndromes due to infiltration of skin and lung epithelium by macrophages and lymphocytes	Huang et al. (1996)
ITGB7	Defects in gut-associated lymphoid tissue formation possibly due to defective lymphocyte attachment	Wagner et al. (1996)
ITGA8	Homozygous nulls die soon after birth due to kidney defects, with ear neuroepithelial deformities	Littlewood Evans and Muller (2000)
Syndecans		
SDC1	Decreased Wnt-1 induced mammary tumorigenesis	Alexander et al. (2000)
SDC3	Feeding behavior defects due to altered hypothalamic functions (predominantly neural)	Reizes et al. (2001)
SDC4	Homozygous mutants display delayed healing of skin wounds and defective angiogenesis	Echtermeyer et al. (2001)
Glypicans		
GPC3	Defects in limb patterning, skeletal development, and kidney branching morphogenesis, renal cystic dysplasia, ventral wall defects	Cano-Gauci et al. (1999), Grisaru et al. (2001), Paine-Saunders et al. (2000)
Dystroglycan		
DAG1	Early embryonic lethality associated with defects in the extra-embryonic basement membrane	Williamson et al. (1997)
DAG1	Conditional mutants with homozygous deletion in the brain display congenital muscular dystrophy syndromes	Moore et al. (2002)
DAG1	Conditional mutants with homozygous deletion in skeletal muscle display muscle regeneration defects	Cohn et al. (2002)

Note: The *Homozygous Mutant Phenotype* column shows the phenotype of mice carrying a targeted homozygous null mutation of the indicated gene, unless otherwise indicated to be a conditional mutation in a certain tissue or a heterozygous mutation.

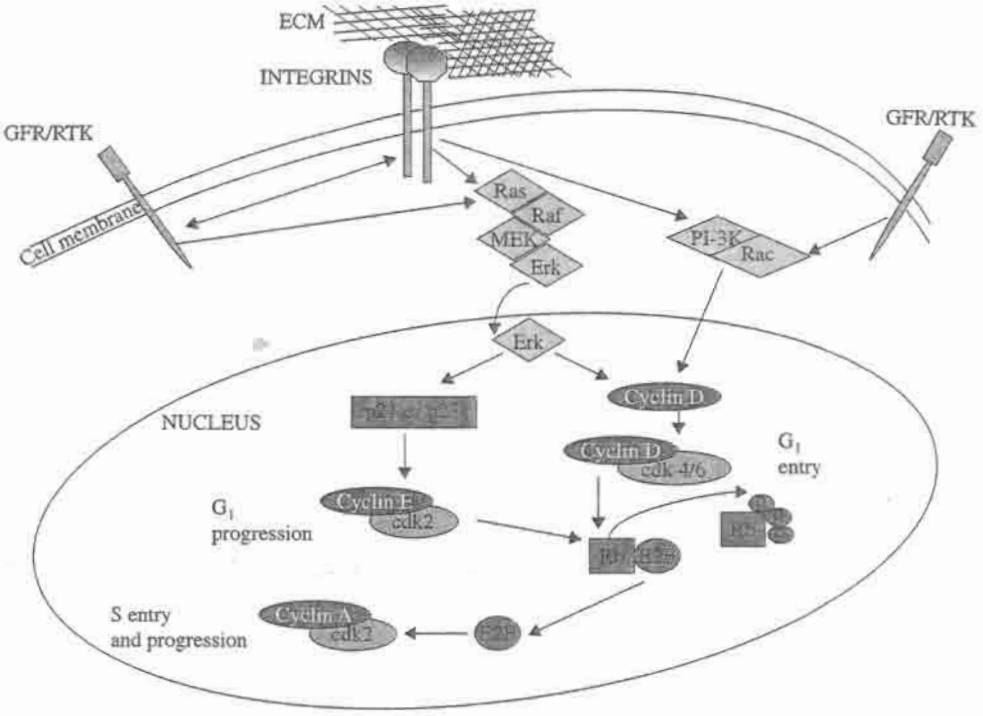


Figure 9.3. ECM-initiated signals regulate G_1 and S phase entry and progression. Sustained activation of Erk via integrin-mediated and/or RTK initiated activation of the MAPK pathway results in translocation of Erk into the nucleus. Erk regulates cyclin D1 expression positively and p21 and p27 negatively. Activation of cyclin D1-cdk4/6 complexes allow G_1 entry. Progression through G_1 is controlled by cyclin E-cdk2. Cyclin E-cdk2 activation requires downregulation of p21 or p27, which is accomplished by Erk-mediated signaling as well. Activation of cyclin E-cdk2 and of cyclin D-cdk4/6 free up the E2F transcription factor by phosphorylating Rb. E2F is needed for transcription of cyclin A. Thus S phase entry and progression that is dependent on cyclin A-cdk2 activation is also regulated by ECM, since downstream targets of cyclin D1 activation and p21 or p27 inactivation include cyclin A. In addition to the MAPK pathway, PI-3K or Rac can also upregulate cyclin D1 transcription through activation of JNK via Akt (a downstream target of PI-3K) or via JNK (a downstream target of Rac).

EFFECTS OF CELL-ECM INTERACTIONS ON CELL CYCLE PROGRESSION IN CULTURED CELLS

Overview

For most cells derived from multicellular organisms, proliferation ex vivo requires simulating at least part of the cellular microenvironment within the tissue. Many nonmalignant cells derived from solid, multicellular organs require adhesion to a substratum in order to proliferate. ECM components, such as fibronectin, laminin, and collagen, have been used as thin monolayer coats onto which cells can attach and grow (Ashkenas et al., 1996). Although plastic that is treated to simulate a

charge distribution that allows cellular attachment has been widely used, presence of ECM provides additional biochemical signals that can promote or inhibit cell proliferation (Cambier et al., 2000; Giancotti and Ruoslahti, 1999; Schwartz, 2001). The ECM effect on cell cycle is dependent on cell type, cell-surface receptor composition, as well as the nature of the ECM molecules.

A classic example of positive regulation of growth by ECM, which was later followed by many other examples, is the demonstration that mouse bone marrow cells, when cultured on a complex ECM derived from marrow, exhibit a dramatically increased ability to proliferate, compared to the controls (Campbell et al., 1985). Negative regulation of growth by ECM in nontumorigenic adherent cells is perhaps best demonstrated by use of three-dimensional (3D) cultures of laminin rich reconstituted basement membrane (lrBM). For example, mammary epithelial cells plated in 3D lrBM proliferate for only a limited number of divisions, arrest growth, and form differentiated colonies resembling tissue structures in vivo, in both shape and function (Fig. 9.4) (Petersen et al., 1992), and specific integrins such as $\alpha v\beta 8$ exert negative growth control in epithelial cells (Cambier et al., 2000). Loss of dependence on adherence or acquisition of anchorage-independent growth (as measured by growth in soft agar) usually accompanies transformation to malignancy. Interestingly, tumorigenic counterparts of cell types that differentiate within 3D ECM cultures exhibit loss of growth regulation as well as their ability to differentiate in response to ECM-mediated signals in the same assay.

Control of Cell Proliferation by Integrin Signaling

ECM-mediated signals that are transmitted via integrins are generally involved in controlling events in G_1 phase progression. Of the G_1 phase cyclins and CDKs (cyclin D, cyclin E, CDK2 and CDK4 and their inhibitors), integrin signaling has the most significant effect on the induction of cyclin D1 and repression of the CDK inhibitors p21 and p27 (Assoian 1997; Roovers and Assoian 2000) (Fig. 9.3). Signaling through integrins is bi-directional (Bissell et al., 1982; Chen et al., 1994). ECM signals are transmitted through integrins to their downstream effectors that regulate expression and activity of the cell cycle regulators in the nucleus. For the rest of this review we call these events ECM-to-nucleus signaling. Growth signals from the nucleus reciprocally regulate the extracellular levels and binding potential of ECM molecules and integrins by regulating their structure and function. We refer to such events as nucleus-to-ECM signaling. While we have focused on integrins as the main transducers of such bi-directional flow of information, or dynamic reciprocity, between the nucleus and cell surface receptors, this phenomenon is also observed for growth factor, cytokine, and other receptors.

ECM-to-Nucleus Signaling. In response to ECM signaling, integrins and many associated proteins cluster and associate with the cellular cytoskeleton to promote filament assembly or disassembly, followed by an intricate series of signaling events that result in changes in a number

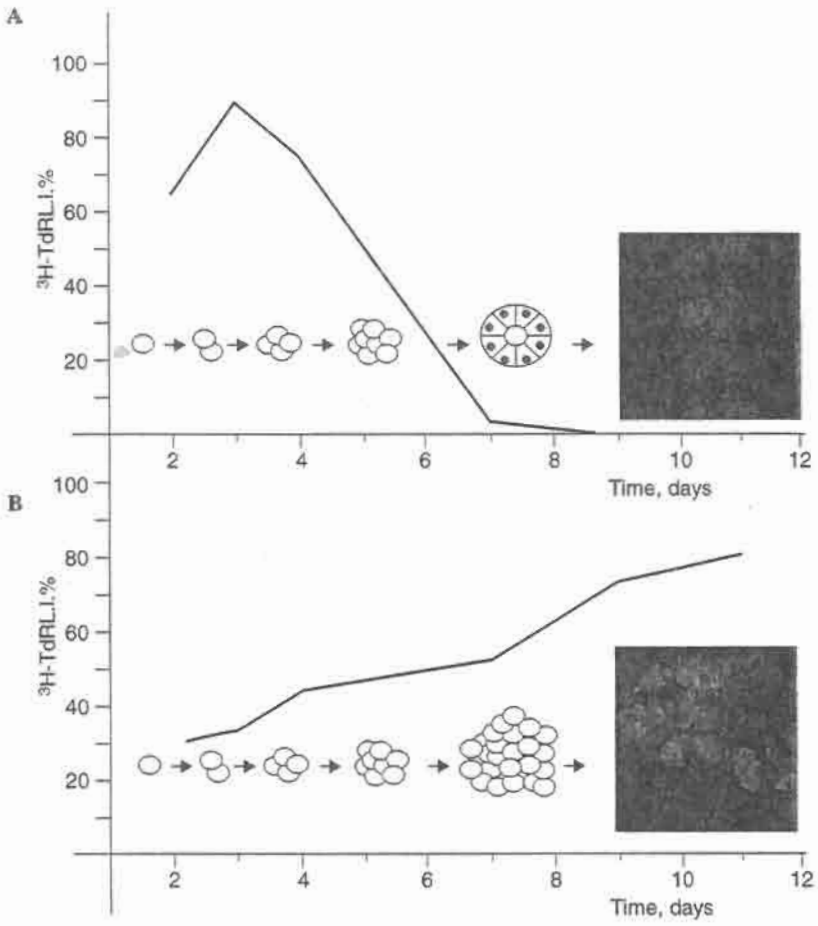


Figure 9.4. Nontumorigenic and tumorigenic mammary epithelial cells differ in their ability to proliferate and differentiate in lrBM. Tritiated tymidine incorporation was measured as a function of time in primary "normal" or nontumorigenic mammary epithelial cell lines **(A)**, and in tumorigenic lines **(B)**. Initially both normal and tumorigenic cells are able to divide. However, normal cells growth arrest and differentiate **(A)**, but tumorigenic cells continue proliferating **(B)** (Reprinted with modifications by permission of Cancer Biology from Weaver et al. 1995). (See color insert.)

of nuclear responses including transcription of cell cycle regulators (Fig. 9.1A). The overview below describes the main components of such signaling events, each of which is a potential point of regulation for cell cycle progression.

Reorganization of actin or keratin filaments into large stress fibers produces signals that feed back into the integrin clusters and causes enhanced integrin clustering and increased binding to the ECM. This region of clustering and signaling is called either a focal adhesion if actin reorganization is involved or a hemidesmosome if the keratin cytoskeleton is involved as in the case of $\alpha 6\beta 4$ signaling (Jones et al., 1998; Nievers

et al., 1999). Although for the rest of this section we focus on focal adhesion-mediated signaling, similar principles also apply to hemidesmosome-mediated signaling. Integrin clustering can further be affected by lateral association of integrins with other membrane proteins, such as caveolin-1 and urokinase plasminogen activator (uPAR), an ECM degrading enzyme (Wary et al., 1996; Wei et al., 1999). Integrin clustering activates some protein tyrosine kinases, including focal adhesion kinase (FAK), Src-family kinases, Abl, as well as a serine-threonine kinase called integrin-linked kinase (ILK) (Giancotti and Ruoslahti, 1999; Wary et al., 1996, 1999). First, interaction of the cytoplasmic tail of integrins with cytoskeletal proteins such as talin and paxillin results in recruitment of FAK to the nascent focal adhesions (Chen et al., 1995; Lewis and Schwartz, 1995; Miyamoto et al., 1995; Parsons et al., 1994; Schaller et al., 1995). This is followed by autophosphorylation of FAK, creating a binding site for Src homology 2 (SH2) domain proteins (Schaller et al., 1994; Schlaepfer et al., 1994). The SH2 domain kinase then phosphorylates a number of focal adhesion proteins, including cytoskeletal proteins such as paxillin and tensin, docking proteins such as $p130^{CAS}$ that recruits adapter proteins such as Crk and Nck (Vuori et al., 1996). FAK can activate phosphoinositide 3-OH kinase (PI 3-kinase) either through activation of Src or directly (Chen et al., 1996). The interaction of Src and FAK can be reciprocal; that is, Src can phosphorylate FAK at the same tyrosine that is autophosphorylated by FAK. This creates a binding site for the adapter complex containing Grb2 and Ras guanosine 5′-triphosphate exchange factor mSOS (Schlaepfer et al., 1994). These signals are transmitted to the MAPK pathway through activation cascade of Ras, Raf, MEK, and ERK sequentially. The MAPKs are also downstream targets of growth factor receptor tyrosine kinases, providing a link between integrin and growth factor receptor signaling. The downstream effects of MAPK signaling include controlling cyclin D1 expression (needed for cell cycle entry), as well as controlling integrin and ECM molecule expression in a feedback loop (nucleus-to-ECM signaling).

Another feedback loop is observed between mitotic signals and FAK. FAK is further phosphorylated on serine when cells enter mitosis. This results in dissociation of FAK from Src and $p130^{CAS}$ (Yamakita et al., 1999). Loosening of focal contacts may allow the cells to decrease adhesion to the substratum and help them divide and spread out. A parallel pathway of integrin-mediated activation starts with activation of a Src family kinase, Fyn, by some integrins. In this pathway a membrane-bound receptor (e.g., caveolin-1) acts as an adapter linking the integrin alpha subunit to Fyn. When ECM binding activates integrins, Fyn becomes activated and its Src homology 3 (SH3) domain interacts with Shc, which in turn phosphorylates it on tyrosine. This targets Shc for the adapter GrbB2-mSOS complex. The SOS complex then transduces signals to the MAPK pathways through Ras, Raf, MEK, and ERK (Schlaepfer et al., 1994), which in turn induce changes in nuclear events that result in enhanced cell proliferation.

Both activation of FAK and Shc (and perhaps other yet unidentified molecules) can contribute to the Ras-ERK MAPK signaling cascade (Howe et al., 2002; Hughes et al., 1997). In some cell types Shc is responsible for the high-level activation of ERK, following cell adhesion to a substratum. FAK most likely has a more significant role in sustaining the activation signal (Pozzi et al., 1998; Wary et al., 1999). Both activation of FAK and Shc can be regulated positively and negatively by tyrosine phosphatases. Some examples include receptor-type protein phosphatase alpha and cytosolic phosphatase SHP-2, which remove the negative regulatory phosphate in Src kinases and therefore amplify both FAK and Shc signaling (Oh et al., 1999; Su et al., 1999). PTP-PEST and PTP-1B are examples of cytoplasmic tyrosine phosphatases that dephosphorylate $p130^{CAS}$; and this inhibits some of the downstream signals of FAK (Garton et al., 1997; Garton and Tonks, 1999; Liu et al., 1998). The phosphatases themselves can be regulated by signals that are integrin-ECM activated. For example, PTP-PEST is anchored to the endoplasmic reticulum and needs to be recruited to the focal adhesions. This occurs when integrin-mediated adhesion activates a protease called calpain, which in turn cleaves the PTP-PEST extension and anchors it to the endoplasmic reticulum, allowing the phosphatase to localize to focal adhesions (Rock et al., 1997). Since phosphatases can have multiple specificities, they can affect cell proliferation by regulating two related pathways. For example, PTEN (a tumor-suppressor gene-encoded protein) dephophorylates PI 3-kinase generated inositol lipids (Li et al., 1997; Maehama and Dixon, 1998; Myers et al., 1998; Stambolic et al., 1998). PTEN can also dephosphorylate FAK and Shc and suppress integrin signaling. Inhibition of PI 3-kinase results in downmodulation of Ras Raf Erk (MAPK) signaling (Gu et al., 1998; Tamura et al., 1998) (Fig. 9.1A). This, in turn, can downmodulate active integrin expression. Therefore a phosphatase like PTEN can inhibit focal adhesion formation via multiple mechanisms. Inhibition of adhesion, as well as down-regulation of the PI 3-kinase survival pathway, can cooperate to reduce the cell's ability to proliferate.

Nucleus-to-ECM Signaling. This can manifest itself in multiple forms (Fig. 9.1B). In many instances, proliferation-related signals that alter nuclear functions result in altered functional expression of cell surface receptors or of the ECM components produced by the cell, thereby changing both the ECM composition surrounding the cell and its ability to respond to the changed microenvironment.

For example, the MAPK-dependent differentiation and growth arrest of PC12 cells is accompanied by up-regulation of $\alpha 1\beta 2$ integrin expression, which helps maintain an elongated morphology via its collagen/laminin interactions (Rossino et al., 1990). A similar nucleus-to-ECM signaling event is observed in erythroleukemia cells that are dependent on MAPK for differentiation; MAPK-mediated growth arrest and differentiation in these cells is accompanied by up-regulation of receptor $\alpha IIb\beta 3$ (Woods et al., 2001). Furthermore expression of some ECM components is dependent on the MAPK-initiated differentiation. Therefore growth arrest related changes in the nucleus result both in

increased ligand concentration for integrin binding and in expression of the integrin receptors (Jones et al., 1999).

An interesting mechanism by which the proliferation status of cells results in nuclear signals that manifest themselves in the form of altered cell-ECM interactions comes from the studies of the Ets transcription factor PEA3 (polyome enhancer activator 3). PEA3 is a direct downstream target of ERK and is essential for the transcriptional activation of many integrins, including αIIb and αv integrins. PEA3 also plays a significant role in the expression of proteases that degrade ECM, such as uPA (urokinase plasminogen activator), collagenases, and stromelysin-1 (Boudreau and Jones, 1999; Crawford and Matrisian, 1996; Wasylyk et al., 1991).

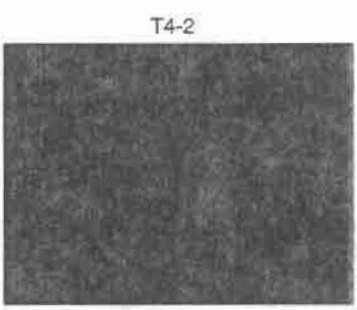
It is intriguing to note that MAPK activation, when it reflects a change in proliferation, can also lead to suppression of high affinity ligand binding of many integrins including β1, β3, and α6, by a mechanism that does not involve transcriptional or translational regulation of integrins or their ligands directly. In a screen for suppressors of integrin activation, H-Ras and its effector kinase Raf-1 were identified as negative regulators of integrin activation (Hughes et al., 1997). H-Ras inhibited the activation of integrins with three distinct alpha and beta subunit cytoplasmic domains. Suppression was independent of both transcription and protein synthesis. Furthermore suppression correlated with activation of the ERK MAP kinase pathway. Therefore the effect of ECM/adhesion on cell proliferation via integrin activation is tightly interconnected with proliferation signals that alter nuclear events. Such nuclear signals regulate integrin composition, affinity of integrins for their ligands, and the composition of the ligands as demonstrated by the transcriptional effects on ECM molecules themselves, as well as on the expression of ECM degrading enzymes.

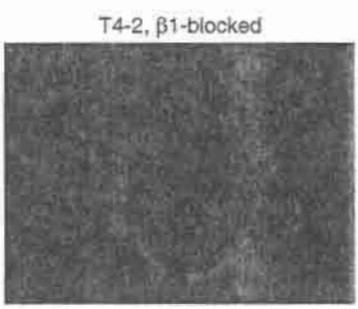
Cooperation of Integrins and Growth Factor Receptors. Multiple mechanisms exist for crosstalk between integrin-mediated and growth factor/receptor tyrosine kinase (RTK) signaling that result in a cumulative effect on cell proliferation (Fig. 9.3). Following integrin activation by ECM, some RTKs are recruited to the focal adhesion complexes. In various cell types, epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR) have been found recruited to focal adhesion complexes (Miyamoto et al., 1996; Plopper and Ingber, 1993; Plopper et al., 1995). This recruitment results in phosphorylation and activation of growth factor receptors and their downstream targets (Miyamoto et al., 1996). Cell adhesion-mediated signals can also affect stability of growth factor receptors as exemplified by an increase in the number of PDGF receptors by blocking ubiquitin-mediated degradation of the receptor following adherence to a fibronectin substratum (Baron and Schwartz, 2000). The downstream MAPK cascade of Ras, Raf, MEK, and ERK can also be differentially affected by adhesion-mediated signaling. There are reported instances where activation of Raf and its downstream targets were promoted by adhesion, but Ras activation was not (Lin et al., 1997;

Roovers and Assoian, 2000). Furthermore integrin-mediated adhesion can be necessary for activation of MEK by Raf and maintenance of active ERK requires adhesion (Howe et al., 2002; Renshaw et al., 1999; Roovers and Assoian, 2000; Roovers et al., 1999). Pathways that are sensitive to both integrin and RTK-initiated signals include the Rac pathway. Growth factor induction of Rac is integrin dependent as demonstrated by observations that RTKs can activate Rac in suspended cells, but the activated Rac is not targeted to the plasma membrane and does not interact and activate its downstream targets such as PI 3-kinase and Akt (del Pozo et al., 2000; Khwaja et al., 1997).

It should be noted that the crosstalk between integrins and RTKs is not only dependent on the presence or absence of integrin-mediated adhesion but also on the nature of the substrata. Experiments with mammary epithelial cells grown either as monolayers on 2D plastic substratum or in 3D laminin-rich reconstituted basement membrane (lrBM) suggest that the composition of the substrata has profound consequences for integrin and growth factor receptor crosstalk. Tumorigenic mammary epithelial cells are unable to growth arrest in 3D lrBM but their normal counterparts stop growth and differentiate into in vivo like structures called acini (Petersen et al., 1992; Weaver et al., 1995) (Fig. 9.4). However, blocking cell-ECM interactions by inhibiting β1 integrin or inactivating EGF receptor signaling in tumorigenic cells in 3D lrBM results in reacquisition of an ability to growth arrest and differentiate (Wang et al., 1998; Weaver et al., 1997) (Fig. 9.5A and B). In 3D lrBM, inhibiting one receptor (β1 integrin or EGFR) results in down-regulation of the expression of the other (EGFR or β1 integrin), but this is not the case when cells are grown in monolayer 2D cultures (Fig. 9.5C). Conversely, both EGFR (ErbB1) and ErbB2 can induce increased proliferation in MCF-10A cells in monolayer, but in 3D lrBM, once the acini are formed, only ErbB2 results in uncontrolled proliferation that fills the acinar lumen with cells and ErbB1 has no effect (Muthuswamy et al., 2001). Taken together with the effect of the MAPK pathway on nucleus-to-ECM signaling that can determine integrin composition, integrin affinity, and ECM molecule composition (see above), these observations suggest that the role of context should be taken into account when interpreting signaling events.

Cell Cycle Regulatory Targets of Integrin-Mediated Signaling. Integrin-mediated signaling can induce either cell cycle progression or exit from the cell cycle followed by differentiation. Positive regulation of cell proliferation by integrins has been observed in cell types that are dependent on adhesion for cell cycle entry. The most common and most highly studied role of integrin signaling on promoting cell cycle entry is in promoting progression to G_1 and the G_1/S transition. The G_1 phase is also controlled by growth factor signals; therefore the crosstalk between integrins and growth factors can manifest itself in the form of co-regulation of cell cycle progression. Although overcoming G_2/M arrest can contribute to cell cycle progression, the role of ECM signaling on the G_2/M transition has not been given much attention. One reason for





C

	3D lr BM				2D plastic		
	S1	fr4-2	T4-2 "treated"		T4-2	T4-2 "treated"	
Inhibitor Added:	–	–	β1 Integrin Inhibitor	EGFR Inhibitor	–	β1 Integrin Inhibitor	EGFR Inhibitor
β1 Integrin total levels	+	+++	+	+	+++	+++	+++
EGFR total levles	+	++++	+	+	++++	++++	++++
EGFR* activated	+	++++	+	+	++++	++++	+

Figure 9.5. Reverted tumorigenic mammary epithelial cells exhibit crosstalk between β1 integrin and EGFR in 3D lrBM but not in 2D monolayer cultures. Treatment of tumorigenic human mammary epithelial cells by inhibitory antibodies against β1 integrin or EGFR results in reversion to differentiated acinar phenotype as shown by re-establishment of basal α6β4 integrin expression. (Reprinted with permission of *Journal of Cell Biology* from Weaver et al., 1997) **(A)** α6 integrin is mislocalized in tumorigenic T4-2 cells (orange). (See color insert.) **(B)** α6 integrin is relocalized to the basal side after tumorigenic T4-2 cells are reverted to a differentiated, nondividing structure in lrBM by treating with inhibitory β1 integrin antibodies (orange). **(C)** Inhibiting β1 integrin in T4-2 cells results in downregulation of the level and activity of EGFR to levels similar to what is observed for non-tumorigenic S1 cells when T4 is grown in 3D lrBM but not in monolayer 2D cultures. Likewise inhibition of EGFR results in β1 integrin down-regulation in 3D but not in 2D. (Reprinted with permission of *Cancer Research* from Bissell et al., 1999).

this is that several studies have shown that only G_1 phase is subject to control by the ECM (and growth factors) for the cell types and the substrata studied (Fang et al., 1996; Sherr, 1994; Zhu et al., 1996). Therefore more thorough studies using a larger number of cell types and substrata are needed to determine if ECM signaling under normal growth conditions may control G_2/M transition. In addition, whether ECM plays a role in the G_2/M progression when cells are arrested in response to external or internal stimuli, such as DNA damaging agents, has not yet been determined.

The mammalian cell cycle is controlled by cyclins, cyclin-dependent kinases (cdks) that are activated by cyclins, cdk inhibitors, and cyclin activators. There are many cdks that function at different stages of the cell cycle. Progression through G_1 phase is modulated by the binding of cyclin D family (D1, D2, D3) to cdk4 or its homolog cdk6 (cdk4/6). Cyclin E binding to cdk2 is also involved in G_1 phase progression. Binding of cyclin A to cdk2 is required for progression through S phase. Activated Cdk4/6 and cdk2 are required for phosphorylation of the retinoblastoma protein (Rb), which activates transcription of genes regulated by E2F family of transcription factors. One of the functions of E2Fs is to regulate expression of cyclin A and therefore S phase entry (Weinberg, 1995). This finalizes the known points of cell cycle regulation initiated by adhesion or growth factors. Progression through G_2/M phase of the cell cycle is activated by binding of cyclin B to cdk1 (= cdc2) (Assoian, 1997; Heichman and Roberts, 1994; Sherr, 1994); however, G_2/M transition does not appear to be one of the main points of regulation initiated by ECM or growth factors. Progression through G_1 or G_2/M can be inhibited by either the INK4 family (p15, p16, p18, and p19) or the p21 family (p21cip1, p27kip1, and p53kip2) of cdk inhibitors. The INK4 inhibitors bind and inhibit cdk4/6, resulting in G_1 arrest. The p21 family of cdk inhibitors can bind to, and inhibit, either cdk4/6 or cdk2, preventing progression to G_1 or S phases of the cell cycle.

The effect of ECM-mediated cell cycle control is mainly manifested by regulation of G_1 and S phase progression for both positive and negative regulatory functions (Schwartz, 2001) (Fig. 9.3). In cases where integrin-mediated adhesion exhibits a positive effect on cell proliferation, activation of the Ras-Raf-MEK-ERK cascade by either ECM-mediated signaling or by growth factors results in increased cyclin D1 expression or down-regulation of the cdk inhibitors p21 and p27 (Roovers and Assoian, 2000). At least in some cases, ERK activity by itself is not sufficient for cyclin D1 expression, cells need to be attached to a substratum (Weber et al., 1997). For example, in suspended CCL39 cells, which require adhesion for proliferation, sustained ERK activity is not sufficient for cyclin D1 activation (Le Gall et al., 1998). It has been reported that translocation of ERK from the cytoplasm to the nucleus is also adhesion dependent (Aplin et al., 2001; Assoian and Schwartz, 2001). It is indicated that other downstream targets that are regulated by both integrin and growth factor receptor/RTK signaling, such as PI 3-kinase, play a role in the transcriptional activation and stability of cyclin D1 (Danilkovitch et al., 2000; Gille and Downward, 1999; Takuwa et al.,

1999). Cyclin D1 regulation by integrin signaling can also be manifested at the level of translation, at least in some cell types, in a manner that is dependent on activation of Rac, a signaling molecule that is translocated to the plasma membrane via events mediated by integrins (Huang et al., 1998; Schwartz and Assoian, 2001). Therefore, in addition to the MAPK signaling cascade, PI 3-kinase and Rac-mediated events can control the transcription and translation of cyclin D1.

Integrin signaling can also mediate cell cycle progression by regulating the cell cycle inhibitors p21 and p27. These cdk inhibitors are normally upregulated in early G_1 phase, but their levels drop as cells progress through G_1 to allow exit from G_1 and entry into the S phase. Induction of p21 in adherence-dependent cells is strongly regulated by ERK activity in early G_1 and the down-regulation of p21 in late G_1 is impaired when integrin signaling is inhibited. However, involvement of ERK with p21 appears to be indirect and through the small GTP binding protein Rho. Regulation of Rho activity by cytoskeletal changes, such as induction of actin stress fibers, has been well studied; however, the impact of integrin signaling on Rho remains somewhat controversial. One reason for the controversy is the difficulty of separating the effects of ECM on cell shape (known to induce Rac) from its effects on integrin signaling. A recent report indicates that activation of the downstream effector of integrin signaling, FAK, can negatively regulate Rho activity (Ren et al., 1999, 2000; Schwartz and Assoian, 2001). Effects of Rho-mediated regulation of cdk inhibitors is likely to turn out to be a cell type, cell surface receptor, and ECM-specific effect, as are many of the signaling cascades described above.

Dependence of cell cycle regulation by ECM on cell type, receptor composition, and substratum identity is best demonstrated by the observations that integrins can also regulate cell cycle progression negatively. For example, expression of $\alpha v\beta 8$ results in increased expression of the cdk inhibitor p21 in a lung carcinoma cell line that has lost $\alpha v\beta 8$ with subsequent growth arrest when grown on a monolayer of vitronectin, an $\alpha v\beta 8$ ligand (Cambier et al., 2000). Many lung tumors have lost their $\alpha v\beta 8$ and introduction of this integrin to tumorigenic lung epithelial cells results in loss of tumorigenicity in nude mice (Cambier et al., 2000).

CONCLUSION

A large number of studies on the effect of ECM on proliferation of cultured cells, data on tissue-specific diseases that are associated with specific ECM signaling components, and knockout mice studies provide clues that control of cell proliferation within a tissue is dependent on the nature of the ECM and its receptors in the tissues. A more comprehensive understanding of contribution of ECM to the tissue-specific regulation of cell cycle progression will require more systematic approaches, including conditional knockouts in different tissues as well as development of culture systems that better mimic physiological tissue environment.

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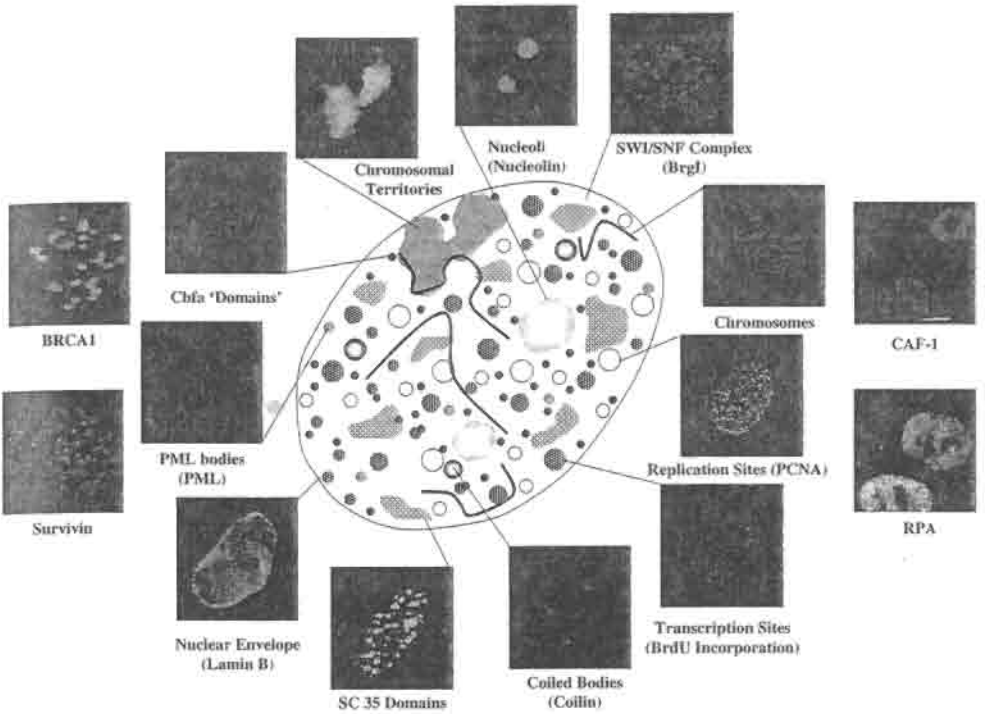

Figure 2.1. Subnuclear compartmentalization of nucleic acids and regulatory proteins into specialized domains. See text for full caption.

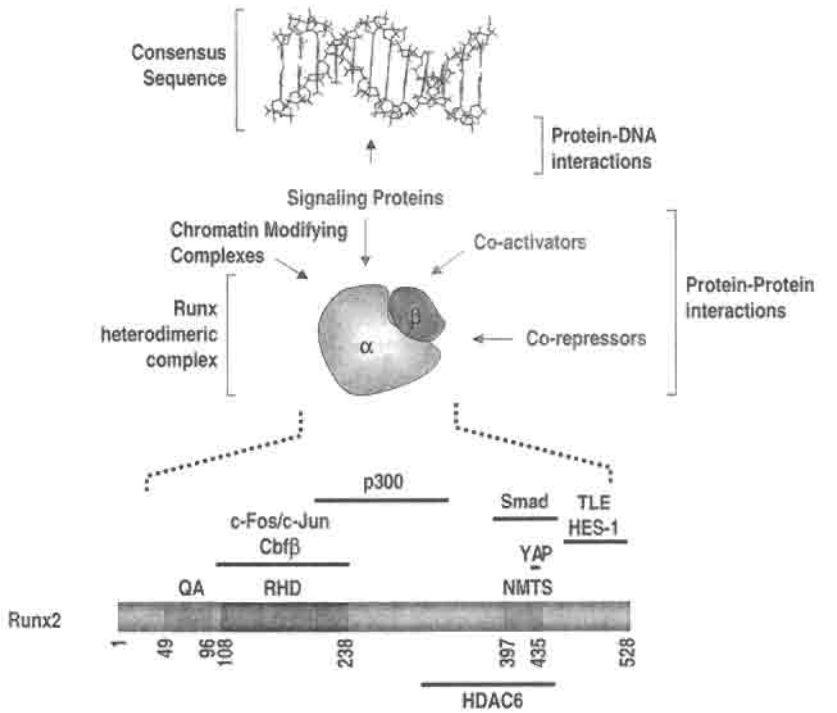

Figure 2.3. Scaffolding nuclear proteins: A mechanism of specificity in gene regulation. See text for full caption.

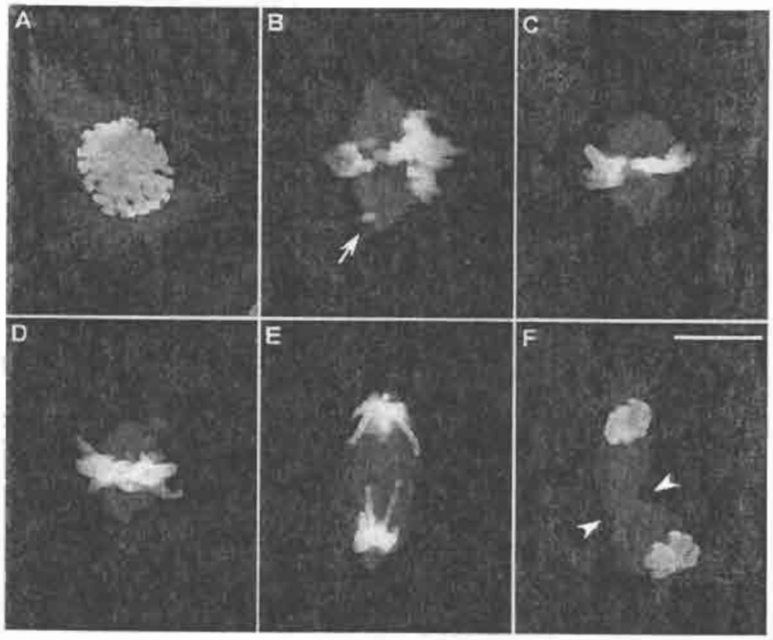

Figure 6.2. Gallery of fluorescent micrographs depicting glutaraldehyde-fixed and lysed PtK_1 cells in various stages of mitosis. See text for full caption.

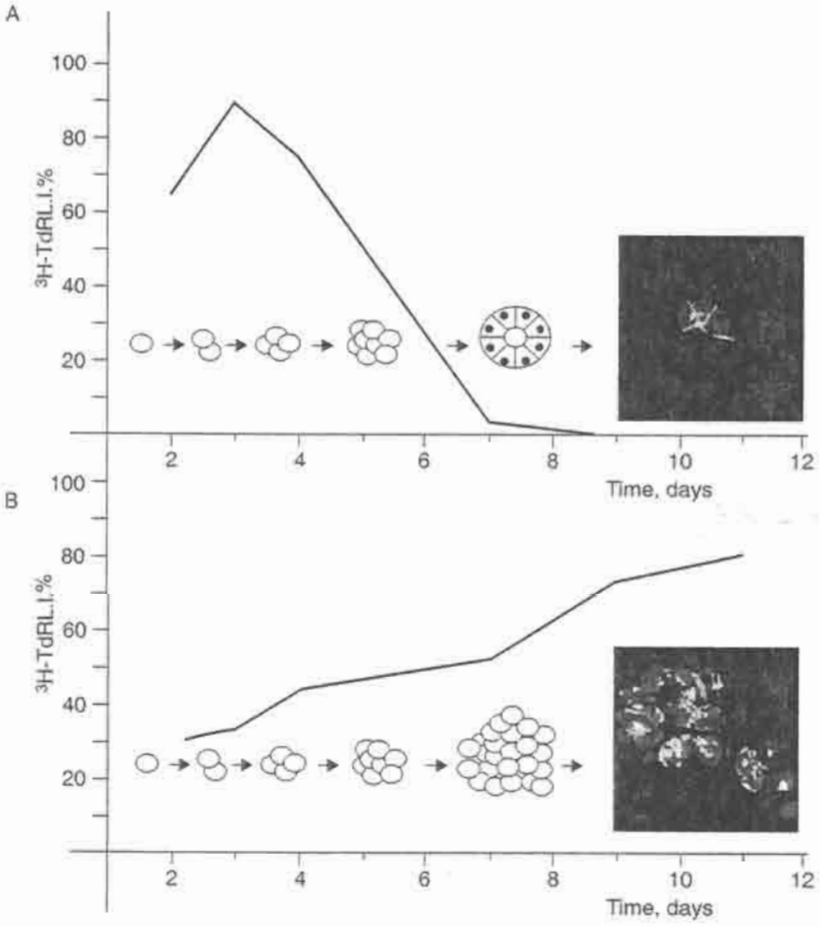

Figure 9.4. Nontumorigenic and tumorigenic mammary epithelial cells differ in their ability to proliferate and differentiate in lrBM. See text for full caption.

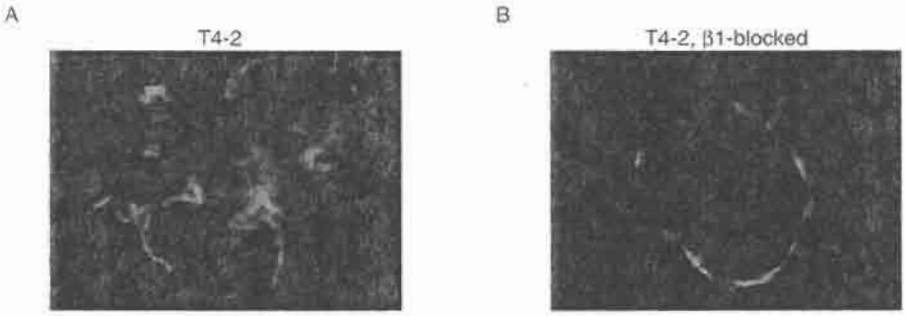

Figure 9.5. Reverted tumorigenic mammary epithelial cells exhibit crosstalk between β1 integrin and EGFR in 3D lrBM but not in 2D monolayer cultures. See text for full caption.

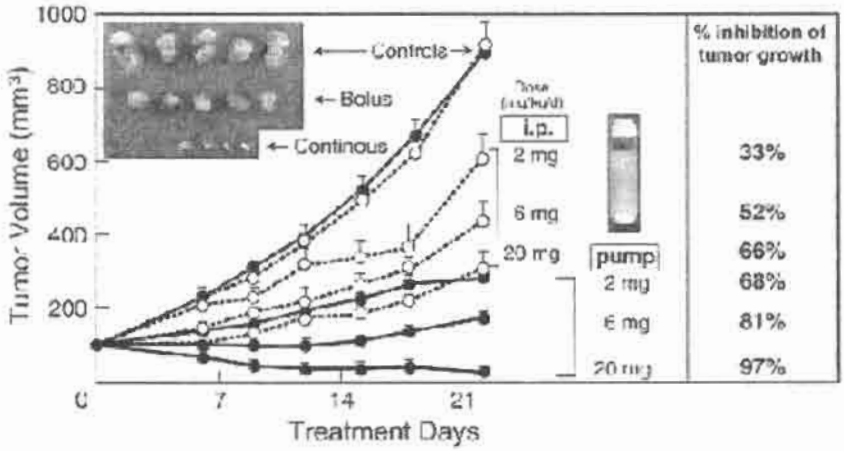

Figure 10.1. Continuous versus bolus administration of human endostatin to SCID mice bearing human pancreatic cancer that is $p53^{-/-}$. See text for full caption.

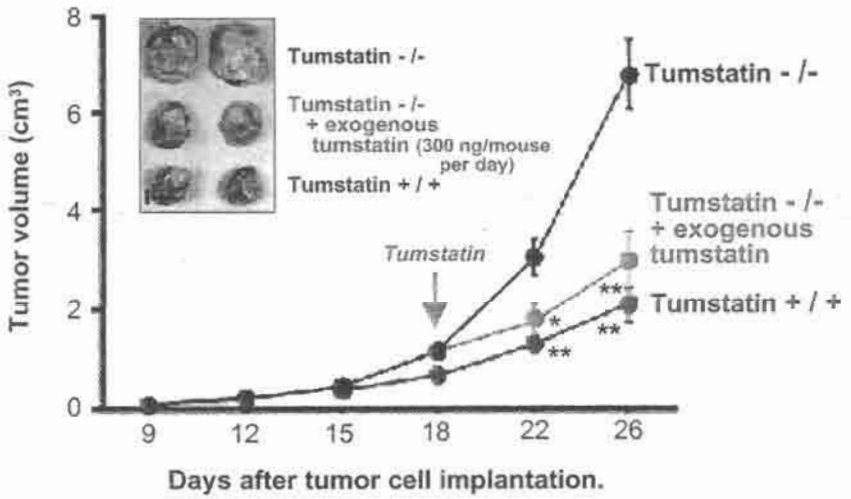

Figure 10.4. In mice depleted of the endogenous angiogenesis inhibitor tumstatin, tumors grow 300% to 400% more rapidly than in wild-type mice. See text for full caption.

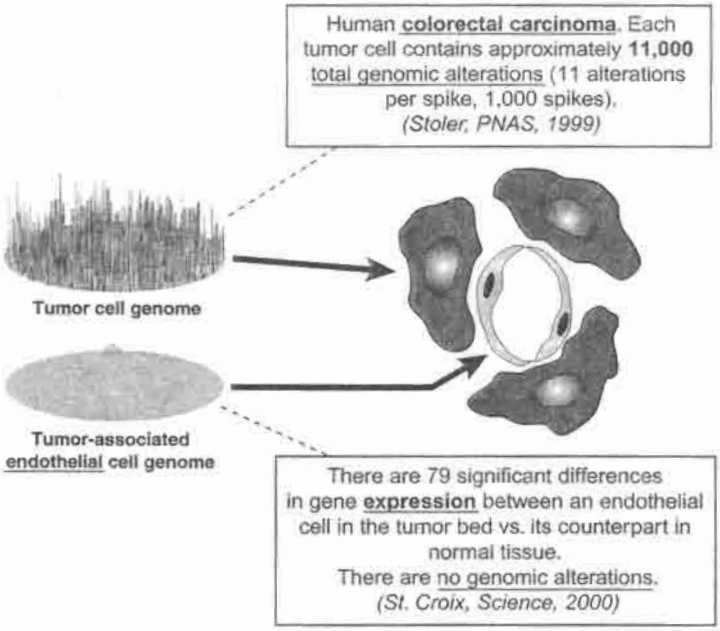

Figure 10.8. Tumor cells are genetically unstable and contain thousands of genomic alterations. See text for full caption.

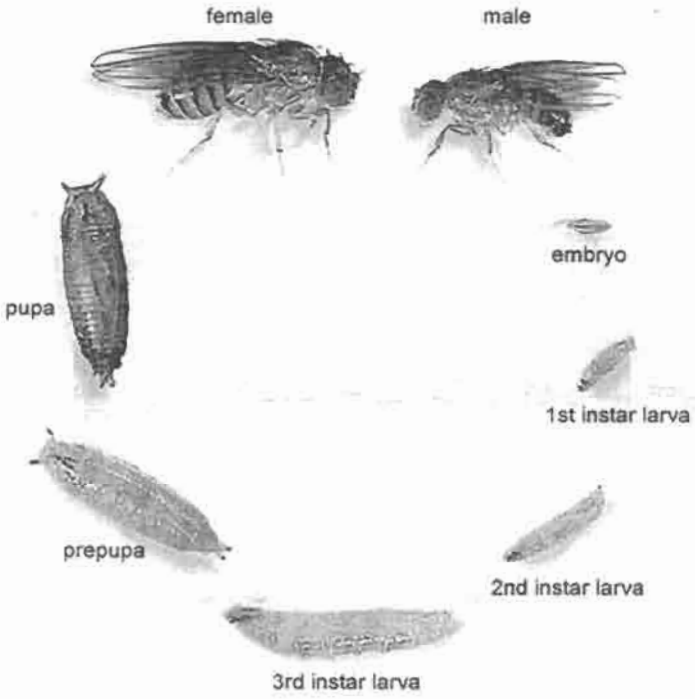

Figure 11.1. Developmental stages of the fruit fly *Drosophila melanogaster*. See text for full caption.

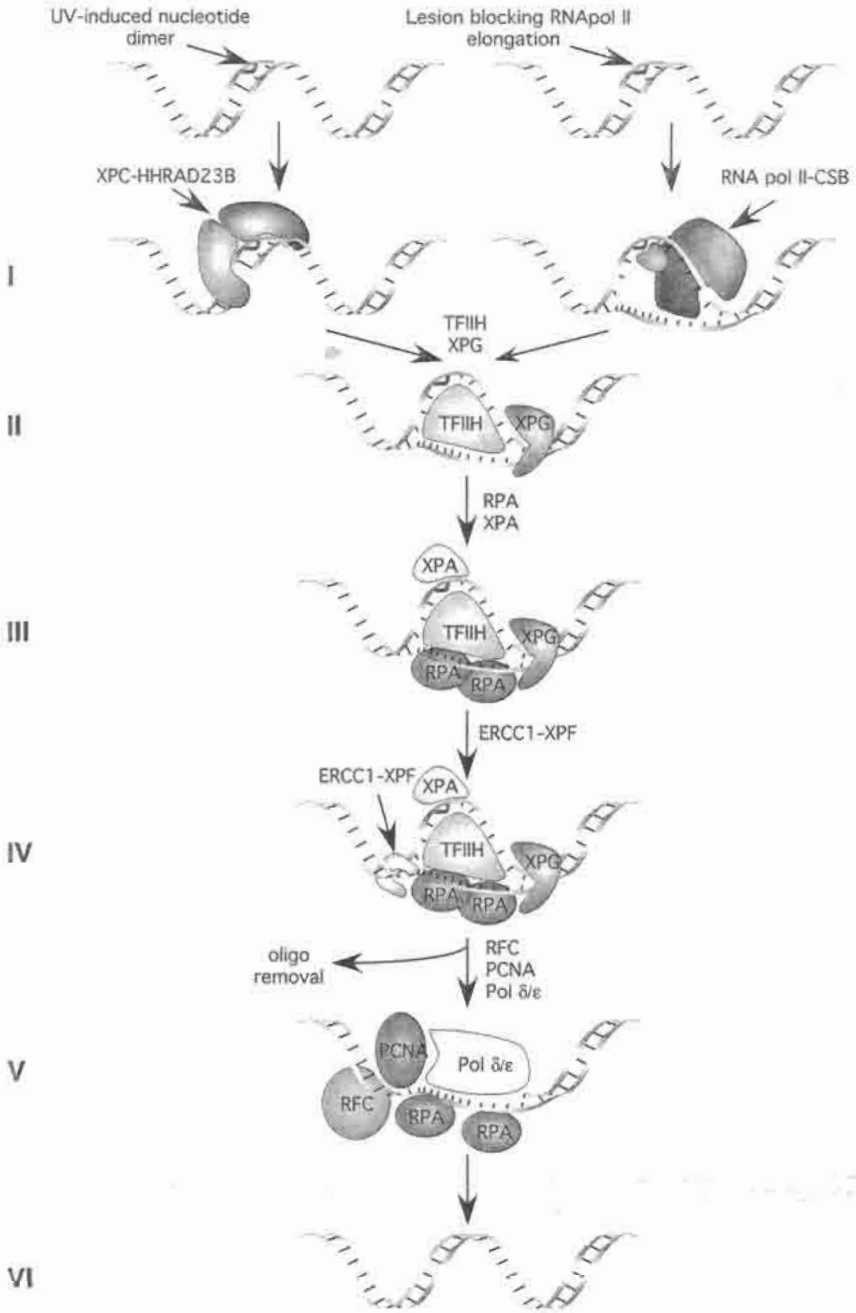

Figure 16.4. (B) Two subpathways exist in nucleotide excision repair, global genome repair, and transcription coupled repair. See text for full caption.

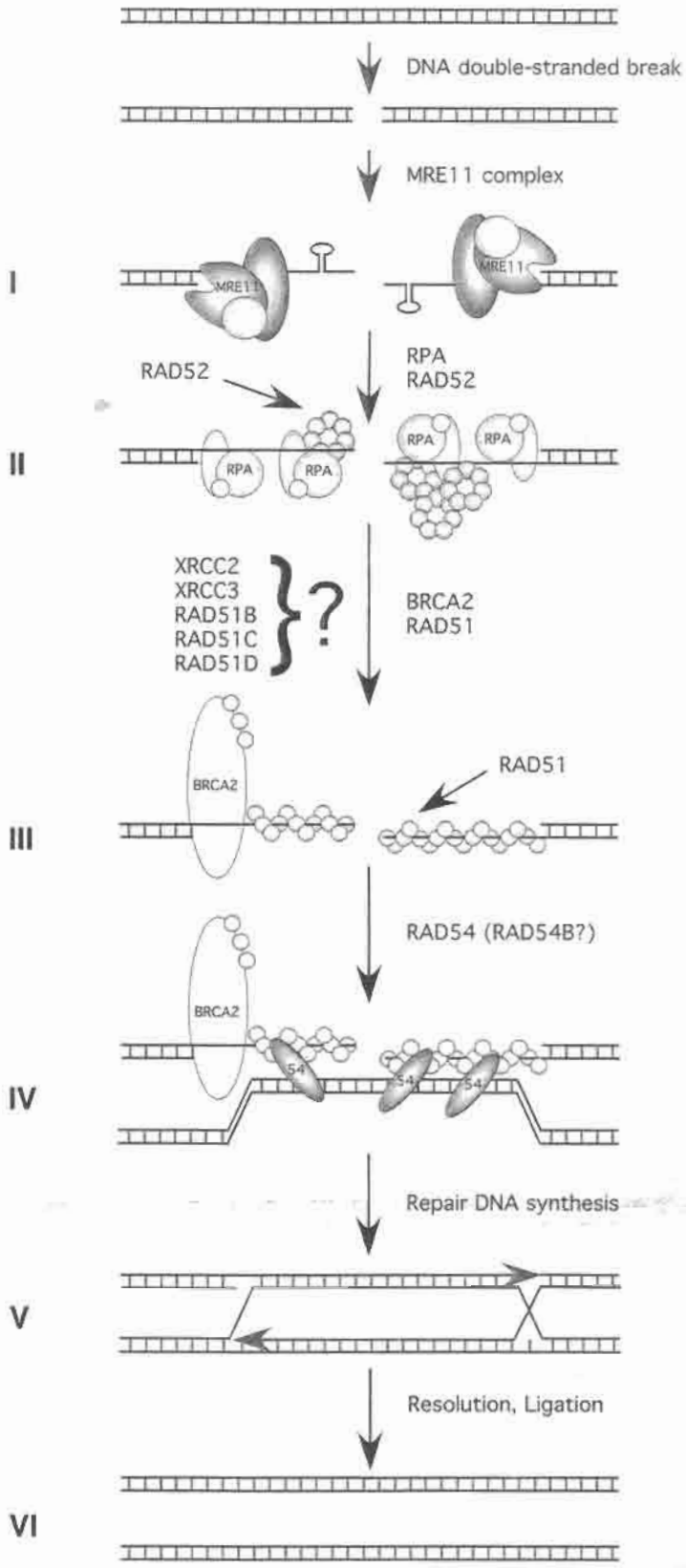

Figure 16.8. Schematic representation of the homologous recombination mechanism. See text for full caption.

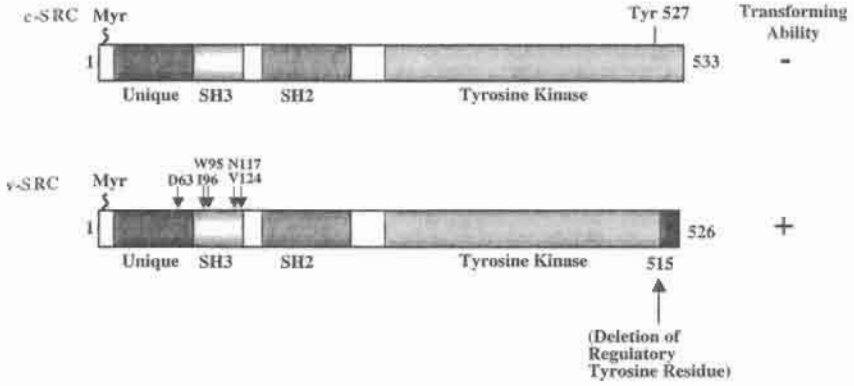

Figure 17.2. Activation of the Src oncoprotein. See text for full caption.

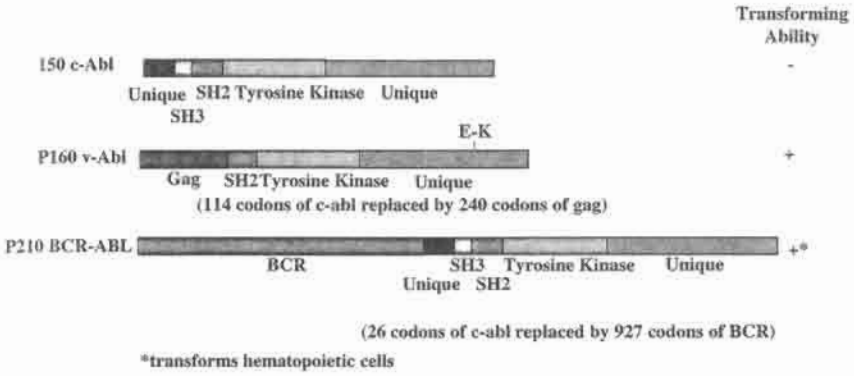

Figure 17.3. Activation of the Abl oncoprotein. See text for full caption.

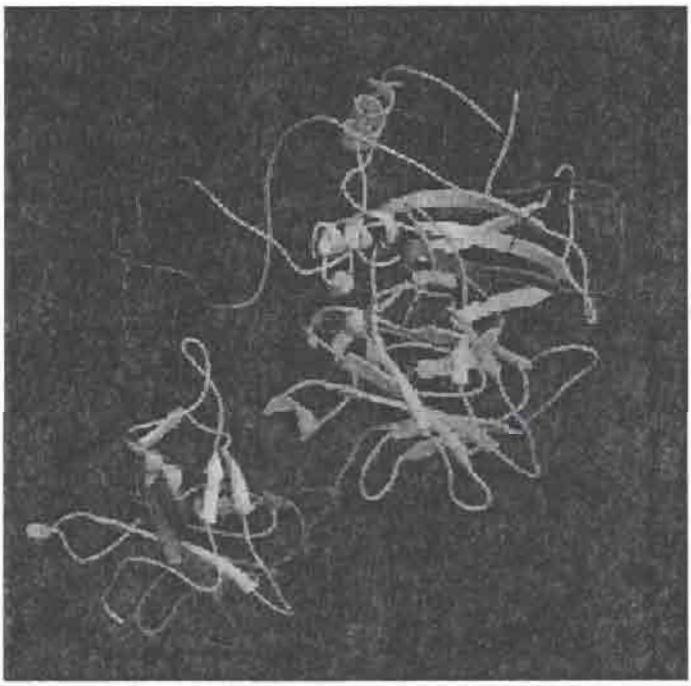

Figure 19.6. Structure of wild-type p53 bound to DNA. Protein Data Bank ID: 1TUP (see Web Resources).

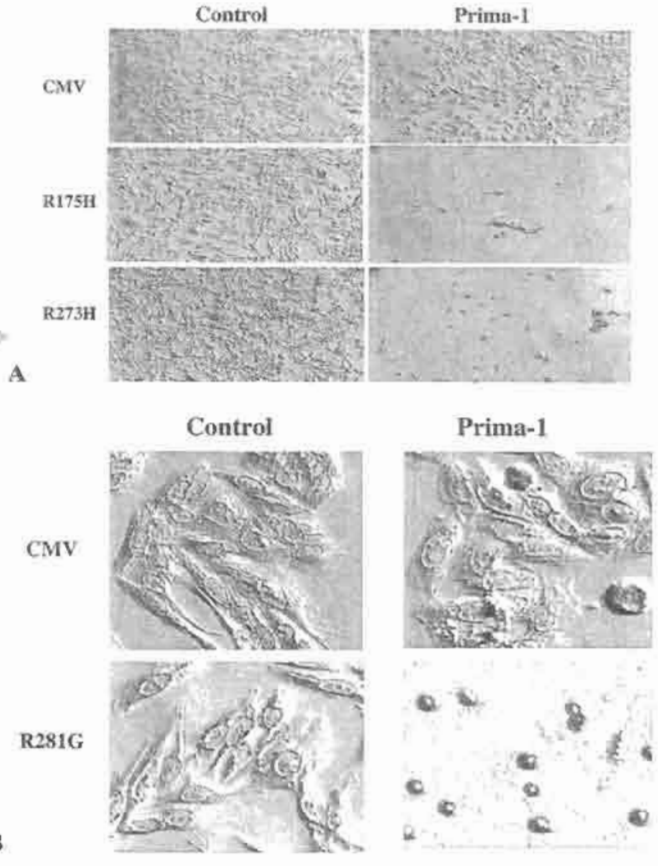

Figure 19.7. Prima-1 reactivates mutant p53. (**A**) Restoration of wild-type p53 activity to mutant p53 by Prima-1 in mouse 10(3) cells. Murine (10)3 fibroblasts lacking endogenous p53 were engineered to express only the selectable marker (CMV) or either the human mutant p53-R175H or R273H. Cells were grown under normal culture conditions (control) or treated with Prima-1 (10μM) for 48 hours and stained for morphological analysis. Note that cells lacking p53 maintained viability after Prima-1 treatment (upper right panel), whereas cells expressing mutant p53 underwent apoptosis (middle and lower right panels) (unpublished data). (**B**) Restoration of wild-type p53 activity to mutant p53 by Prima-1 in Saos-2 cells. Human osteosarcoma Saos-2 cells lacking endogenous p53 were engineered to express only the selectable marker (CMV) or human mutant p53-R281G. Cells were grown under normal culture conditions (Control) or treated with Prima-1 (75μM) for 48 hours and stained for morphological analysis. Note that cells lacking p53 maintained viability after Prima-1 treatment (upper right panel) whereas cells expressing mutant p53 underwent apoptosis (lower right panel) (unpublished data).

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